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Entrapment of enzymes and nanoparticles using biomimetically synthesized silica†‡

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Entrapment of enzymes and nanoparticles using biosilicification reactions.

The successful use of enzymes for applications in catalysis and sensors is dependent on the host material used for immobilization of the enzymes. Sol-gel and functionalized mesoporous silica are widely used methods for the immobilization of enzymes.[1] These methods have some inherent limitations, for example, harsh sol-gel processing conditions result in the loss of enzyme activity. In nature, diatoms are considered as a paradigm for the controlled production of nanostructured silica.[2] Diatoms are single-celled algae that form cell walls composed of a nanoporous network of silica nanoparticles. Proteins identified in sponges and diatoms have been shown to be responsible for the formation of siliceous structures.[3,4] The R5 repeat unit peptide [H_2N-SSKKSGSYSGSKGSKRRIL-COOH] of the silaffin protein from *Cylindrotheca fusiformis* has been shown to catalyze the formation of silica under ambient conditions *in vitro*.[4] Similarly, silica-binding peptides identified from a combinatorial peptide library have also been demonstrated to precipitate silica particles from silicic acid.[5] Interestingly, the silica-precipitating peptides become entrapped during the generation of the silica matrix.[6] This observation has led us to explore the use of the biosilicification reaction as a new route for the entrapment of an exogenously added enzyme in a silica matrix.[7] This initial study indicated that the biosilicification reaction might serve as an alternative method for the entrapment of enzymes using more biologically compatible reaction conditions.

Here we demonstrate the utility of the biosilicification reaction for the entrapment of a variety of enzymes and inorganic nanoparticles. The entrapment process is a one-pot procedure wherein silica synthesis and enzyme entrapment occur simultaneously. The R5 peptide condenses the water-soluble alkoxide (silicic acid) to form a network of fused silica particles.[4] The enzyme or nanoparticle to be entrapped is mixed with the R5 peptide prior to the addition of the alkoxide precusor (0.1 M tetramethoxysilane) in sodium phosphate buffer pH 7.5. The reaction is carried out at room temperature. The resulting silica precipitate was collected, washed several times in buffer and stored in buffer or as a dry powder at room temperature. The silica precipitate with the entrapped enzyme was analyzed by scanning electron microscopy (SEM). As expected, a network of large aggregates of fused silica particles was observed (Fig. 1A). The activity of the silica-entrapped enzymes was determined. As shown in Fig. 1B, the immobilized enzymes, catalase or horseradish peroxidase (HRP) retained their enzymatic activity and had comparable activities to those of the free enzymes. Initial results obtained using Michaelis–Menten enzyme kinetic analysis indicated that the entrapped HRP retained comparable activity to that of the free soluble enzyme.

Addition of the enzymes after the biosilicification reaction yielded little or no detectable enzyme activity after washing the silica matrix, confirming that this is not an absorption-driven process. For example, catalase or HRP added to the silica matrix and then washed several times in sodium phosphate buffer containing 0.2% Tween-20 resulted in little or no detectable enzyme activity. This suggests that the enzyme activity observed with the silica-entrapped enzymes is due to entrapment of the enzyme within the silica matrix.

We confirmed that catalase is entrapped within the silica matrix by dissolving the silica matrix in 1 M sodium hydroxide for 10 min at 37 °C, to release the entrapped catalase enzyme. The released enzyme was recovered in the supernatant solution and was detected using denaturing polyacrylamide gel electrophoresis (SDS-PAGE). Coomassie blue staining of the polyacrylamide gel revealed the presence of a protein band with a molecular weight of *ca*. 60 kDa, the expected molecular weight for catalase (Fig. 1C). This result confirms that the enzyme was entrapped within the silica matrix. The entrapped enzymes can be stored at room temperature in a dry state for several days with no significant loss in enzyme activity (Fig. 1D). Similar observations of increased thermal stability have also been noted using traditional sol-gel entrapment procedures.[8] Further optimization of our entrapment procedure should produce higher catalytic activity.

Inorganic nanoparticles can also be entrapped in silica using the method described here. We introduced magnetic cobalt platinum (CoPt) or streptavidin conjugated CdSe@ZnS (QD605) nanoparticles into the biosilicification reaction. CoPt nanoparticles were synthesized as previously described.[9] Similar to the results obtained using enzymes, we were able to demonstrate the entrapment of CoPt and CdSe@ZnS nanoparticles within the silica matrix. The

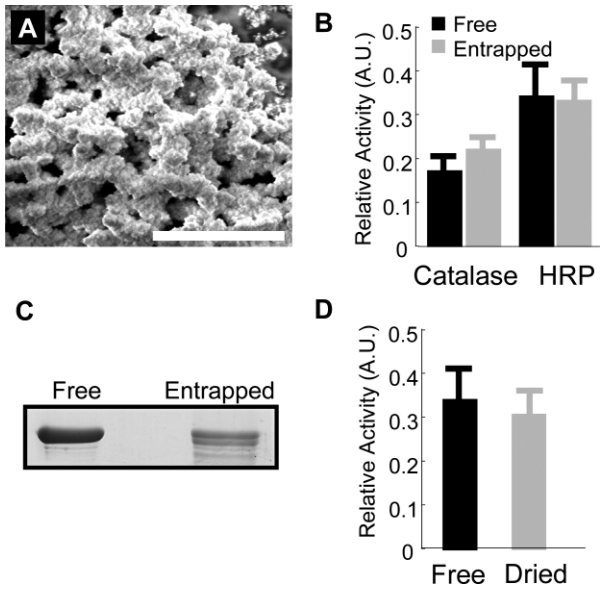

Fig. 1 Activity of the silica entrapped enzymes. (A) SEM micrograph of silica immobilized catalase, scale bar 5 μm. (B) The activity of entrapped catalase and horseradish peroxidase (HRP) compared to the free enzyme. (C) Coomassie blue stained SDS-PAGE gel demonstrating that the enzyme is entrapped within the silica matrix, for comparison the free enzyme is shown. (D) The dried silica entrapped catalase retains enzyme activity.

† Electronic supplementary information (ESI) available: biosilicification reaction, enzyme assays, encapsulation of iron oxide. See http://www.rsc.org/suppdata/cc/b4/b404586f/

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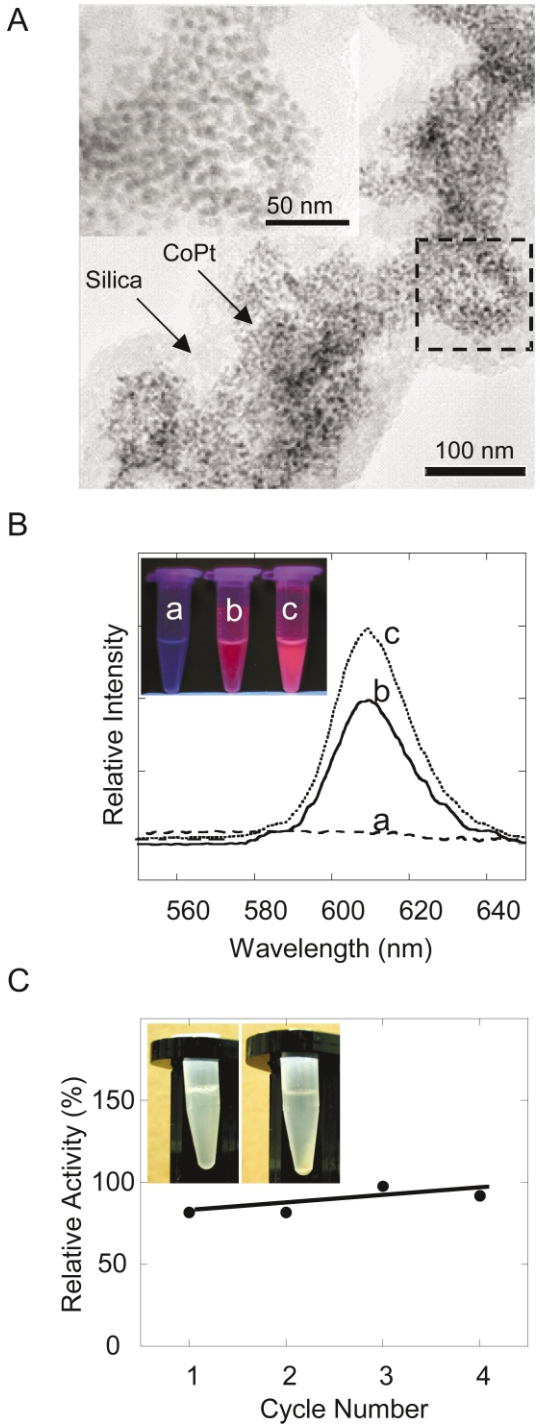

Fig. 2 Encapsulation of inorganic nanoparticles. (A) TEM micrograph of CoPt nanoparticles entrapped within the silica matrix. Inset: High resolution TEM of the boxed region. The average size of the CoPt nanoparticles is 4 nm. (B) Fluorescence emission spectra of the silica matrix (curve a), quantum dots QD605 (curve b) and silica encapsulated QD605 (curve c) when excited at 405 nm. Inset: solutions of biosilica matrix (tube a), QD605 nanoparticles (tube b) and silica entrapped QD605 nanoparticles (tube c) when illuminated with a longwave hand-held UV lamp. (C) Relative activity of the magnetic catalytic silica matrix after repeated use. Iron oxide nanoparticles added along with catalase into the biosilicification reaction with R5 peptide to obtain enzyme/nano-magnetic entrapped silica matrix. Activity was assayed using hydrogen peroxide as a substrate. The magnetic catalyst was separated from the reaction product using a magnet. Separation of the enzyme/nano-magnetic entrapped silica matrix before and after 5 min incubation using a magnetic separation stand (inset).

CoPt nanoparticles are entrapped within the silica matrix as shown in the transmission electron microscopy (TEM) micrographs in Fig. 2A.

The silica entrapped CdSe@ZnS nanoparticles were characterized by measuring the emission spectrum (Fig. 2B). Both free and entrapped CdSe@ZnS nanoparticles exhibit similar emission profiles. It has not escaped our notice that addition of magnetic nanoparticles to the entrapped enzyme–silica matrix will facilitate separation of the entrapped enzyme from the product.[10] Iron oxide nanoparticles were entrapped along with the enzyme in a silica matrix using the biosilicification reaction. This results in the reusability of the entrapped enzyme after magnetic separation (Fig. 2C). The advantages of using biomimetic silica for entrapment are the relatively benign reaction conditions used for entrapping enzymes. In contrast, sol-gel methodologies employ harsh reaction conditions for encapsulation resulting in loss of enzyme activity. Biomolecules entrapped using the method described here can be used in biocatalytic and biosensing applications.

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